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**FERROUS IRON - TURNBULL'S BLUE**

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**PURPOSE:** To detect ferrous ( $\text{Fe}^{2+}$ ) iron in tissues.

**PRINCIPLE:** Tissue sections are treated with an acidic solution of potassium ferricyanide, any ferrous iron present will react to form an insoluble bright blue pigment called Turnbull's blue (ferrous ferricyanide).

**CONTROL:** Routine iron control, which includes an negative.

**TECHNIQUE:** Cut paraffin section  $4\mu$ .

**EQUIPMENT:** Acid clean glassware, non-metallic forceps.

**REAGENTS:**

**0.006N Hydrochloric Acid**

Hydrochloric acid            2.5 ml  
Distilled water                497.5 ml

Mix well. Solution is stable for 1 year.

**CAUTION:** Corrosive acid.

**1% Acetic Acid**

Acetic acid, glacial            1.0 ml  
Distilled water                100.0 ml

Mix well. Solution is stable for 1 year.

**CAUTION:** Avoid contact and inhalation.

**Potassium Ferricyanide  
Staining Solution:**

Potassium ferricyanide        0.4 gm  
Hydrochloric acid, 0.006N    40.0 ml

Prepare fresh, just before use.

**CAUTION:** Avoid contact and inhalation.

**Nuclear-Fast Red:  
See Retic**

**SAFETY:** Wear gloves, goggles and lab coat. Avoid contact and inhalation.

Hydrochloric acid; target organ effects on reproductive system and fetal tissue. Irritant to skin eyes and respiratory system.

Potassium ferricyanide; Low toxicity as long as it is not heated, it will release cyanide gas.

Acetic acid: Irritating to respiratory system. Target organ effects on respiratory system by inhalation. Corrosive.

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Place slides in Potassium ferricyanide staining solution for 1 hour.
3. Wash slides in 1% acetic acid.
4. Counterstain slides in nuclear-fast red for 5 minutes.
5. Rinse well in distilled water.
6. Dehydrate, clear and coverslip.

**RESULTS:**

Ferrous iron	blue
Background	pink-red

**REFERENCE:**

Carson, F, Histotechnology: A Self-Instructional Text, 1st Ed., 1991, pp. 215-16 ASCP Press

Prepared: \_\_\_\_\_ By: \_\_\_\_\_

Approved: \_\_\_\_\_ By: \_\_\_\_\_

PROCEDURE CARD  
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See Reitec

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EXPIRATION: \_\_\_\_\_

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Mix well. Solution is stable for 1 year.

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**NUCLEAR-FAST RED**

DATE: \_\_\_\_\_

TECH: \_\_\_\_\_